

CLAIMS:

1. A genetically stable, transformed *Lemnaceae* plant and progeny thereof.
- 5 2. A transformed *Lemnaceae* plant according to Claim 1, of the genera *Spirodela*, *Lemna* and *Wolffia*.
3. A transformed *Lemnaceae* plant according to Claim 2, being *Spirodela punctata* of strain 8717.
4. An antibiotic resistant transformed *Lemnaceae* plant according to Claims 1 to 3.
- 10 5. A transformed *Lemnaceae* plant according to Claim 4, being resistant to kanamycin.
6. A herbicide resistant transformed *Lemnaceae* plant according to ^{Claim 1} ~~Claims 1 to 3~~.
- 15 7. A transformed *Lemnaceae* plant according to Claim 4, being tolerant to oxynil herbicides, to glyphosate and EPSPS inhibitor herbicides, to glufosinate or to HPPD inhibitors.
8. A transformed *Lemnaceae* plant according to ^{Claim 1} ~~Claims 1 to 7~~, capable of expressing two or more foreign genes.
- 20 9. Use of the plant according to Claim 1, for the production of chemical or biological products.
10. Use according to Claim 9, for the production of polypeptides, proteins, carbohydrates, lipids, alkaloids, pigments or vitamins.
11. A chemical or biological product obtained by the use according to Claim 9 ~~or 10~~.
- 25 12. A method for the stable genetic transformation of *Lemnaceae* plants which comprises: incubating *Lemnaceae* plants and/or tissue with *Agrobacterium* cells containing a transforming DNA molecule, whereby cells in said plant tissue become stably transformed with said DNA.
- 30 13. A method according to Claim 12, wherein the *Agrobacterium* cells are capable of specifically targeting the plant's meristematic tissue.
14. A method according to Claim 13, wherein the *Agrobacterium* cells are

A. tumefaciens strains EHA105, EHA101 and GVE3103.

15. A method according to Claim 12, wherein the *Agrobacterium* cells are capable of targeting wounded regions in the plant.

16. A method according to Claim 15, wherein the *Agrobacterium* is *A. tumefaciens* strains LBA4404 and C58.

17. A method according to Claim 12, wherein during the incubation of the *Lemnaceae* plant tissue with the *Agrobacterium* cells vacuum infiltration is applied.

18. A method according to Claim 12, wherein prior to incubation of the *Lemnaceae* plant tissue with the *Agrobacterium* cells the plant's meristematic zone is exposed by removal of the daughter fronds.

19. A method for the genetic transformation of a plant comprising: cutting the plant into particles of a size such that they still contain undamaged meristematic tissue capable of developing into full plants; incubating said particles with *Agrobacterium* cells containing transforming DNA molecules, whereby said transforming DNA is introduced into meristematic cells in said particles; and producing transformed plants from the transformed meristematic tissue.

20. A method according to Claim 19, wherein the plant is a *Lemnaceae* plant.

21. A method according to Claim 19 or 20, wherein the particles have an average diameter above about 150 μm .

22. A method according to Claim 21, wherein the particles have an average diameter of about 150 μm - 750 μm .

23. A method for the stable genetic transformation of a *Lemnaceae* plant comprising microinjecting *Agrobacterium* cells containing a transforming DNA into the meristematic zone of the plant, whereby the meristemic tissue becomes stably transformed with said DNA.

24. A method according to Claim 23, carried out *in planta*.

25. A method for the *in planta* transformation of *Lemnaceae* plants comprising:

- i. exposing the plant's meristematic zone by removal of the daughter fronds;

- ii. incubating the plant with *Agrobacterium* cells capable of targeting to the meristemic tissue.

26. A method according to Claim 25, wherein the *Agrobacterium* cells are *A. tumefaciens* strains EHA105, EHA101 and GVE3103.

5 27. A method according to ^{Claim 12} ~~any one of Claims 12 to 26~~, wherein the *Agrobacterium* cells are brought into contact, prior or during the transformation method, with a booster medium capable of enhancing the *Agrobacterium* cell's virulence.

10 28. A method according to ^{Claim 12} ~~any one of Claims 12 to 26~~ wherein the transformation process takes place in a media having a pH below about 5.2.

29. A method according to Claim 28, wherein the booster medium further comprises a fresh cell suspension obtained from a dicotyledonous plant.

30. A method according to ^{Claim 28} ~~Claims 28 or 29~~, wherein the fresh cell suspension is at a concentration of 1-10% (w/v).

15 31. A method according to ^{Claim 28} ~~Claims 28 to 30~~, further comprising caffeine at a concentration of 100-500 mg per liter of medium.

32. A method according to ^{Claim 28} ~~any one of Claims 28 to 31~~, wherein the fresh cell suspension of a dicotyledonous plant is obtained from the family of *Solanaceae*.

20 33. A method according to ^{Claim 26} ~~any one of Claims 26 to 32~~, wherein the medium is a plant culture medium having a pH of about 3.5 to 4.2, and comprising 1-10% (w/v) of fresh cell suspension of *Nicotiana tabacum* and 100-500 mg per liter of medium caffeine.

34. A method according to Claim 27, wherein the booster medium comprises a *Lemnaceae* plant extract.

25 35. A method according to Claim 34, wherein the *Lemnaceae* plant extracts are *Spirodela punctata* extracts.

36. A transformed *Lemnaceae* plant obtained by the method of any one of Claims 12 to 35.

30 37. A booster medium for enhancing *Agrobacterium* cell's virulence comprising plant tissue culture at a pH below about 5.2.

38. A booster medium according to Claim 37, further comprising a fresh cell suspension of a dicotyledonous plant.

39. A booster medium according to Claim 38, wherein the fresh cell suspension is at a concentration of 1-10% (w/v).
40. A booster medium according to ~~any of Claims 37 to 39~~, further comprising caffeine at a concentration of 100-500 mg per liter of medium.
41. A booster medium according to ~~any of Claims 37 to 40~~, wherein the fresh cell suspension is of plants from the family of *Solanaceae*.
42. A booster medium according to ~~any of Claim 37 to 41~~, comprising plant growth medium at a pH of above 3.5 to 4.2, 1-10% (w/v) of fresh cell suspension of *Nicotiana tabacum*, and 100-500 mg per liter of medium caffeine.
43. A booster medium for enhancing *Agrobacterium* cell's virulence comprising an extract from *Lemnaceae* plants.
44. A booster medium according to Claim 41, comprising extracts of *Spirodela punctata* plants.
45. A method for maintaining morphogenetic *Lemnaceae* calli for long-periods of time comprising culturing the calli in a medium having a low level of sucrose.
46. A method according to Claim 45, wherein the sucrose level is less than 1.5%.
47. A method for the regeneration of plants from calli wherein the plant's growth medium has sucrose levels below 1.5% and comprises: B5, minerals and organic compounds.
48. A method for the production of highly regenerative calli, wherein the calli's growth medium has sucrose levels below 1.5% and comprises B5, minerals and organic compounds.
49. A method according to Claim 47 ~~or 48~~, wherein the growth media further comprises phytohormones.
50. A method for the production of highly regenerative calli, wherein the calli's growth medium has sucrose levels below 1.5% and comprises B5, minerals, organic compounds and selection agents.
51. A method according to Claim 50, wherein the selection agents are selected from the group consisting of: antibiotics, herbicides, or metabolic inhibitors.

- Article 34
52. A method for the production of stable transformed plants, wherein the growth media has sucrose levels below 1.5% and comprises B5, minerals and organic compounds.
53. A method according to Claim 52, wherein the growth media further comprises phytohormones.
- a 54. A method of production of a product of interest, comprising growing a transformed *Lemnaceae* according to one of Claims 1 to 8, coding said product in an appropriate culture medium, under conditions enabling the production of said product of interest.
55. The method as claimed in Claim 54, wherein the product of interest is further isolated and purified.
- d 56. A method as claimed in one of Claims 54 or 55, wherein the product of interest is a chemical or a biological product.
57. A method as claimed in Claim 56, wherein the product of interest is selected from the group consisting of polypeptides, proteins, carbohydrates, lipids, alkaloids, pigments or vitamins.
58. A method according to Claim 35, wherein the *Lemnaceae* is *Spirodela*.
59. A method for forming *Lemnaceae* calli by separating between the mother frond and the daughter frond, using a plucking motion.
60. A method according to Claim 47, wherein the plants are *Lemnaceae*.
61. A method according to Claim 60, wherein the plants are *Spirodela*.
- 002200 22762360
- ATA BI

AMENDED SHEET